A STUDY OF THE COTTON FLEA HOPPER, PSALLUS SERIATUS REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES

DISSERTATION

Presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Ohio State University.

BY

REGINALD HENRY PAINTER, B.A., M.A.

L. C.

THE OHIO STATE UNIVERSITY

1926

STANFORD UNIVERSITY LIBRARIES

3781
O37P

UNCLASSED

A STUDY OF THE COTTON FLEA HOPPER, PSALLUS SERIATUS REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES

DISSERTATION

Presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the Ohio State University.

By

Reginald Henry Painter, B.A., M.A.

The Ohio State University

1926

A STUDY OF THE COTTON FLEA HOPPER, PSALLUS SERIATUS REUT., WITH ESPECIAL REFERENCE TO ITS EFFECT ON COTTON PLANT TISSUES[1] [2]

By REGINALD H. PAINTER
Assistant Professor of Entomology, Kansas Agricultural Experiment Station [3]

INTRODUCTION

The cotton flea hopper, *Psallus seriatus* Reuter, which belongs to the family Miridae, has only recently become important as a cotton pest (*5*).[4] The spread through the Southern States of a peculiar form of injury traceable to this insect has been rapid, although the insect itself has been known to be present there for some time. The generalized effect of the attack of the insect on the cotton plant leads one to look for the presence of other factors in addition to simple insect feeding.

During the summer of 1925 an investigation was made of the possibility of the transmission of a plant disease by the cotton flea hopper, and in order to get a definite anatomical basis for future work both with regard to the insect and the injured plant structure the study was made in three parts: (1) A study of the anatomy of the digestive apparatus of the insect; (2) a study of pathological and of healthy plant tissue; and (3) an examination of the fresh material for verification of findings in preserved material and examination of digestive enzymes present.

TECHNIC

The material intended for preservation and later study was collected at Port Lavaca, Tex., and after fixation was preserved in 80 per cent alcohol until used at Columbus, Ohio. Eight different fixing fluids were used. Bouin's, Zenker's, and Petrunkewitsch's fixing fluids were found to be the best for insect material. Bouin's fluid and a formalin-acetic acid-alcohol formula by Chamberlain (*2*) were found to be the best for the plant material. Heidenhain's iron haematoxylin, used by the longer method, and Ehrlich's acid haematoxylin, both counterstained with orange G., were found to be the most satisfactory stains for both plant and insect tissues.

[1] Received for publication July 24, 1929; issued March, 1930.

[2] This paper was presented in August, 1926, as a dissertation in partial fulfillment of the requirements for the degree of doctor of philosophy in the graduate school of the Ohio State University. The work upon which it is based was begun while the author was in the status of temporary field assistant in the Bureau of Entomology, U. S. Department of Agriculture, and completed in 1926 while he was in the status of collaborator of the Bureau of Entomology and graduate student, Ohio State University.

[3] The first experiments were made under the direction of the late Dr. W. D. Hunter, to whom the writer is indebted for many useful suggestions. The writer wishes to thank the members of the faculty of the department of zoology and entomology of Ohio State University, especially Drs. Herbert Osborn, Raymond C. Osburn, and C. H. Kennedy, and Dr. H. C. Sampson, of the department of botany, for valuable assistance and suggestions. Thanks are also due to members of the U. S. Bureau of Entomology, especially to Mr. B. R. Coad and Dr. J. W. Folsom, for making possible the investigation and for the excellent facilities furnished at the Delta laboratory, Tallulah, La., during July, 1926. The writer wishes also to thank Mr. Lawrence Youman, of Columbus, Ohio, for help with the photomicrographs of plant tissue. Most of the microtechnic work in connection with the preparation of slides for study was done by Mrs. R. H. Painter, to whose efforts was due the excellence of the material that was studied. This manuscript was submitted to the Bureau of Entomology for publication Sept. 28, 1926.

[4] Reference is made by number (italic) to "Literature cited," p. 516.

In studying the gross anatomy of these minute insects the following method was used for dissection and found to be successful if a strong enough illumination was used: The insect which had been fixed and stored was removed from the alcohol and allowed to dry a few minutes on filter paper. It was then placed on its side in a small drop of melted paraffin on the bottom of a watch-glass dissecting dish and completely covered with a thin coat of paraffin. The dish was flooded with cold 50 per cent alcohol and placed under the binocular. Scalpels were prepared by cementing a scrap of safety-razor blade in the end of a glass tube. With these it was possible to remove the exoskeleton together with the paraffin and expose the internal organs. The alcohol was then poured off. A drop of Ehrlich's acid haematoxylin was placed on the insect and allowed to stand for about five minutes and was then washed off. Dissection was then resumed under alcohol. This method does not stain entirely through the insect at one time, and it is often necessary to repeat the staining process. The coloring of the insect, a part at a time, is often of advantage. Organs may be stained in this manner and removed for mounting on slides.

Although a number of other hemipterous forms have been studied with regard to their internal anatomy and histology, relatively little is known about the Miridae in this respect.

Dufour (*3*), in 1833, figured and described the anatomy of the digestive tract of two European mirids, but the writer has been unable to find a description of the histology of any member of the family.

STRUCTURE AND HISTOLOGY OF THE ALIMENTARY CANAL AND ITS APPENDAGES

The structure of the mouth parts and salivary apparatus of *Psallus seriatus* is, on the whole, similar to that of other Hemiptera. With regard to homologies of parts the writer has followed the terminology [5] given in a General Textbook of Entomology, by Imms (*6*), and no attempt has been made to study these parts in regard to their relationship to similar parts in other members of the order. Hence the parts will be only briefly described and any structures peculiar to this insect will be noted. The description below applies to the adult except as otherwise mentioned.

MOUTH PARTS

The labium is 4-segmented and is furnished at the tip, as is usual in the order, with sensory hairs and pits. The proximal segment is quite heavily muscled for the bending of this organ at the time of feeding. This musculature, however, has not been worked out in detail. A groove, which is not entirely closed at any point, runs the length of this rostrum, except at its extreme basal end, and contains the stylets. Above the basal end of these mouth parts are the epipharynx and labrum, which are not easily distinguishable from each other. At the base of these is a groove in which the stylets lie.

The stylets consist of the mandibles and maxillae, all of which are hollow except at the extreme tips, and at their bases possess glands and muscular attachments. In the head each pair of stylets

[5] A paper by Snodgrass (*9*), which appeared after this manuscript was submitted for publication, gives a different and probably better interpretation of the parts of the hemipteran head. An explanation of the function of the "retort-shaped organs" described herein is also given in his paper.

is somewhat enlarged and flattened, the mandibles lying above the maxillae. As they pass into the groove of the epipharynx, the mandibles take up a position on each side of the maxillae. The latter possess two grooves on the inner faces which, when opposed to one another, form dorsally the suction canal and ventrally the ejection canal. The former connects with the pharynx, the latter with the salivary duct, opening through the hypopharynx. The stylets themselves are interlocked and grooved (fig. 1, D) and are furnished with ridges which fit into grooves in the ventral plate of the epipharynx where the stylets emerge from the head. At their tips the mandibles are of a heavier structure and are slightly shorter than the maxillae, and, whereas the latter are thin and barbed on their dorsal edge, the former are apparently smooth.

In the attachment of the stylets there are apparently no levers such as are present in some of the other Hemiptera, but each appendage is attached by a pair of muscles, as shown in Figure 1, E and F. In the head the upper and inner pair of stylets are the mandibles. The small dorsal muscle of the mandible attaches to the vertex after passing out just in front of the optic nerve. The ventral muscle passes beneath and to the side of the brain and attaches to the occiput or back wall of the head capsule. (Fig. 1, E.) On the maxillae the muscles are larger; one passes anteriorly, attaching to the maxillary sclerite near its junction with the labrum; the other extends posteriorly, passing beneath and behind the optic nerve and attaching to the occiput just below and to the side of the origin of the ventral mandibular muscle. (Fig. 1, F.)

There is a gland at the base of each stylet which in the adult is small. At the base of the maxilla it is usually L-shaped, extending laterally and dorsally, with the expanded bladelike portion of the stylet forming a chitinous wall to part of the gland; at the base of the mandible it is more pyriform, lying above, and external to, the hollow chitinous rod. The cavities of these glands are continuous with the cavities of the stylets and sometimes show tissue inside the base of the cavities. These glands are apparently the homologues of the "retorten formigen Organen" of German authors.

In the third, fourth, and fifth nymphal stages the stylets are each connected with spiral coils of tissue lying in the prothorax, which are much more like the "retort-shaped organs" of aphids and other Homoptera. These coils and the connections with the stylets are hollow and are lined with a chitinous membrane except at the proximal end in the interior of the coil. This enlarged proximal end and some of the tissue surrounding the coil consist of a compact mass of quite distinct cells with enlarged nuclei. Toward the end of the fifth nymphal stage these structures degenerate entirely, and the glands at the base of the stylets in the adult are all that are left. The details of these structures are not included in this paper, as they apparently have no intimate relation to the digestive secretions of the insect.

BUCCAL GLANDS

Immediately above the place where the maxillae come together to form the suction and ejection canals lie certain interesting cells which may perhaps be best termed the "buccal gland cells." In this region, which probably represents all that is left of the mouth cavity in the Hemiptera, the dorsal plate, a continuation of the dorsal

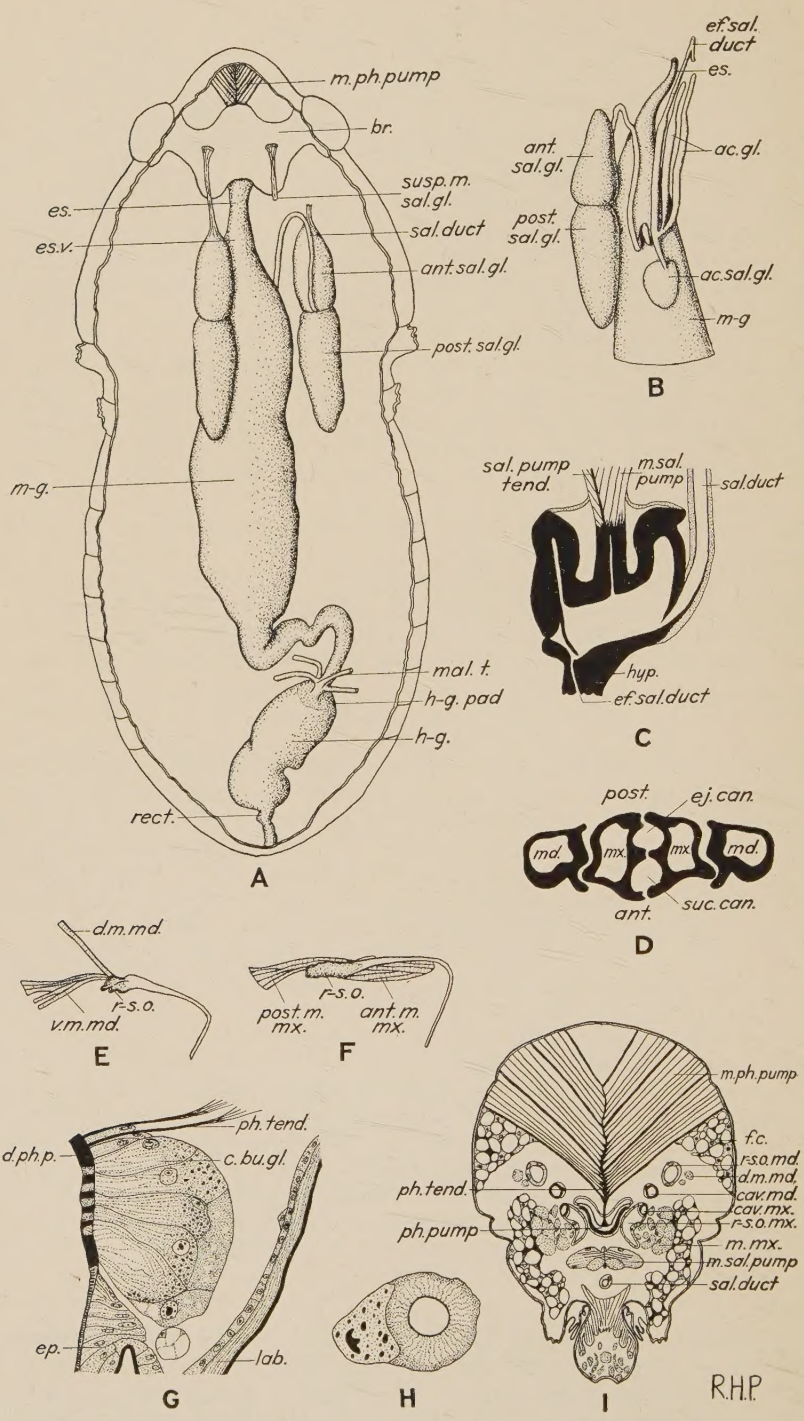

FIGURE 1.—The cotton flea hopper, *Psallus seriatus*. (For explanatory legend see opposite page)

pharyngeal plate, is perforated by two longitudinal rows of five pores each, above which lie the gland cells. A longitudinal section of these structures is shown in Figure 1, G. Near the pores the cytoplasm of these cells is sparse and fibrous; the distal ends of the cells, in which lie the nuclei, are filled with large granules that take haematoxylin stain quite deeply. Several cells containing cytoplasm of quite a different structure lie beyond the distal end of the gland cells proper.

The function of these glands is problematical, and probably they are too small for experimentation. Their secretion, which is of small quantity, mingles with the incoming food and may in some way take the place of the secretion of the salivary glands which, in other insects, open at about this point, since in this insect the salivary glands proper do not communicate directly with the alimentary canal. The buccal gland cells may be sensory in function, as for taste, but the writer has been unable to find a nervous connection of any size; moreover, these cells have the appearance of having the function of secretion.

ALIMENTARY CANAL

For the purpose of description the alimentary canal may be divided conveniently into the fore-gut consisting of the pharynx and the esophagus, the undifferentiated mid-gut separated from it by the esophageal valve, and the hind-gut. Like the mid-gut, the hind-gut shows little or no separation into distinct divisions.

The pharynx extends from the buccal glands back to a point almost beneath the center of the brain. In cross section it is horseshoe shaped and consists of a heavy chitinous ventral plate connected with a lighter dorsal plate by a region thinly chitinized. Surrounding this structure is a very thin layer of small epithelial cells; few or no muscular elements are to be seen, either as circular or longitudinal muscles. Piercing this epithelial layer and attached to the center of the dorsal plate is a comblike structure of erect chitinous tendons. This structure runs the entire length of the pharynx. From their point of attachment on the chitinous tendons, divergent muscles extend to the sides and are fastened to the vertex and front, appearing V-shaped

EXPLANATORY LEGEND FOR FIGURE 1

A.—General anatomy of the alimentary canal and salivary glands, drawn from dissection and sections × 107; *ant. sal. gl.*, anterior salivary gland; *br.*, brain; *es. v.*, esophageal valve; *es.*, esophagus; *h-g.*, hind-gut *h.-g. pad*, hind-gut pad; *m-g.*, mid-gut; *m. ph. pump*, muscles of salivary pump; *mal. t.*, Malpighian tubule *post. sal. gl.*, posterior salivary gland; *rect.*, rectum; *sal. duct*, salivary duct; *susp. m. sal. gl.*, suspensory muscle of salivary gland.

B.—Salivary gland, duct, and accessory gland, drawn from a dissection of a last-stage nymph, × 107; *ac. gl.*, accessory gland; *ac. sal. gl.*, accessory salivary gland; *ant. sal. gl.*, anterior salivary gland; *ef. sal. duct*, efferent salivary duct; *es.*, esophagus; *m-g.*, mid-gut; *post. sal. gl.*, posterior salivary gland.

C.—Longitudinal section of the salivary pump, drawn from an unstained whole mount, × 893; *ef. sal. duct*, efferent salivary duct; *hyp.*, hypopharynx; *m. sal. pump*, muscles of salivary pump; *sal. duct*, salivary duct; *sal. pump tend.*, salivary pump tendon.

D.—Cross section of stylets just after they emerge from the head, × 893; *ant.*, anterior; *ej. can.*, ejection canal; *md.*, mandible; *mx.*, maxillae; *post.*, posterior; *suc. can.*, suction canal.

E.—Base of mandible with retort-shaped organ and muscular attachments, × 481; *d. m. md.*, dorsal muscle of mandible; *r-s. o.*, retort-shaped organ; *v. m. md.*, ventral muscle of the mandible.

F.—Base of maxillae with retort-shaped organ and muscular attachments, × 481; *ant. m. mx.*, anterior muscle of maxillae; *post. m. mx.*, posterior muscle of maxillae; *r-s.o.*, retort-shaped organ.

G.—Longitudinal section of buccal gland cells and pores, × 893; *c. bu. gl.*, cells of buccal gland; *d. ph. p.*, dorsal pharyngeal plate; *ep.*, epipharynx; *lab.*, labrum; *ph. tend.*, pharyngeal tendon.

H.—Cross section of salivary duct, × 413.

I.—Cross section of head just in front of the eyes, × 308; *cav. md.*, cavity of mandible; *cav. mx.*, cavity of maxillae; *d. m. md.*, dorsal muscle of mandible; *f. c.*, fat cells; *m. mx.*, muscle of maxillae; *m. ph. pump*, muscles of pharyngeal pump; *m. sal. pump*, muscles of salivary pump; *ph. pump*, pharyngeal pump; *ph. tend.*, pharyngeal tendon; *r-s. o. md.*, retort-shaped organ of mandible; *r-s. o. mx.*, retort-shaped organ of maxillae; *sal. duct*, salivary duct.

in cross section. (Fig. 1, A and I.) The entire structure may be called the pharyngeal pump.

The esophagus extends from almost beneath the center of the brain, where it attaches to the pharynx proper, to a point near the middle of the prothorax, where it merges into the esophageal valve. In the esophagus the intima is formed as a direct continuation of the dorsal and ventral plates of the pharynx, but being very much thinner it is only barely visible under high-power magnification. The epithelial layer is extremely thin, but its cell walls may still be distinguished in good preparations. A few strands of longitudinal muscle extend the length of the esophagus, but these are very small. The circular muscles, basement membrane, and peritoneal membrane are either absent or too delicate to be visible. Four small muscles support the esophagus just back of the brain. Two tracheal trunks run alongside the esophagus through the circumesophageal connectives.

The esophageal valve is very simple and is well shown in Figure 2, B. At the junction of the mid-gut and fore-gut a ring of one or two cells secretes a peritrophic membrane which is short and disappears entirely only a very short distance below the entrance of the mid-gut. Except for these few cells there is an abrupt change from the structure of the fore-gut to that of the mid-gut.

The mid-gut extends from the esophageal valve into the posterior third of the abdomen with few curves and showing no definite morphological divisions exteriorly. The anterior part is usually much more distended than the posterior part and occupies the bulk of the body space in that region. There is a distinct coil to the right just above the pyloric valve in most specimens. The contents of the mid-gut have been studied and will be described later. Histologically, the mid-gut consists principally of large columnar epithelial cells which vary somewhat in size and shape, according to the distension of the stomach and the age of the cells. A loose lattice work of small strands of circular and longitudinal muscles covers the entire mid-gut, the former being on the inside. The longitudinal muscle strands are relatively few. There seems to be no peritoneal membrane. The enteric epithelium just below the esophageal valve consists of fairly regular cells with large nuclei, each cell with a distal striated hem. Farther down in the stomach wall the cells when they are young and small still possess this hem; as they get older they appear to lose it before discharging their secretory contents. These older cells give off droplike protrusions which extend into the lumen of the gut and later break off and discharge their contents. Relatively few small, young cells have been noticed, so that it is probable that secretion by any one cell is more or less continuous. In the living material the cells of the epithelium are often filled with what appear to be oil globules, which very often are seen to take up green coloring matter, presumably chlorophyll. This same color often appears in the cells of the fat bodies. In preserved material and sections these oil globules show as vacuoles of varying size. There seems to be no correlation between the size of these globules and the age of the cell or the condition of the individual. In many of the cells of some specimens there occur bodies of varying size which stain a deep black in iron haematoxylin. The possible nature of these will be discussed later. The remaining cytoplasm is fairly uniform. There seem to be no distinct differences or divisions in the kinds of

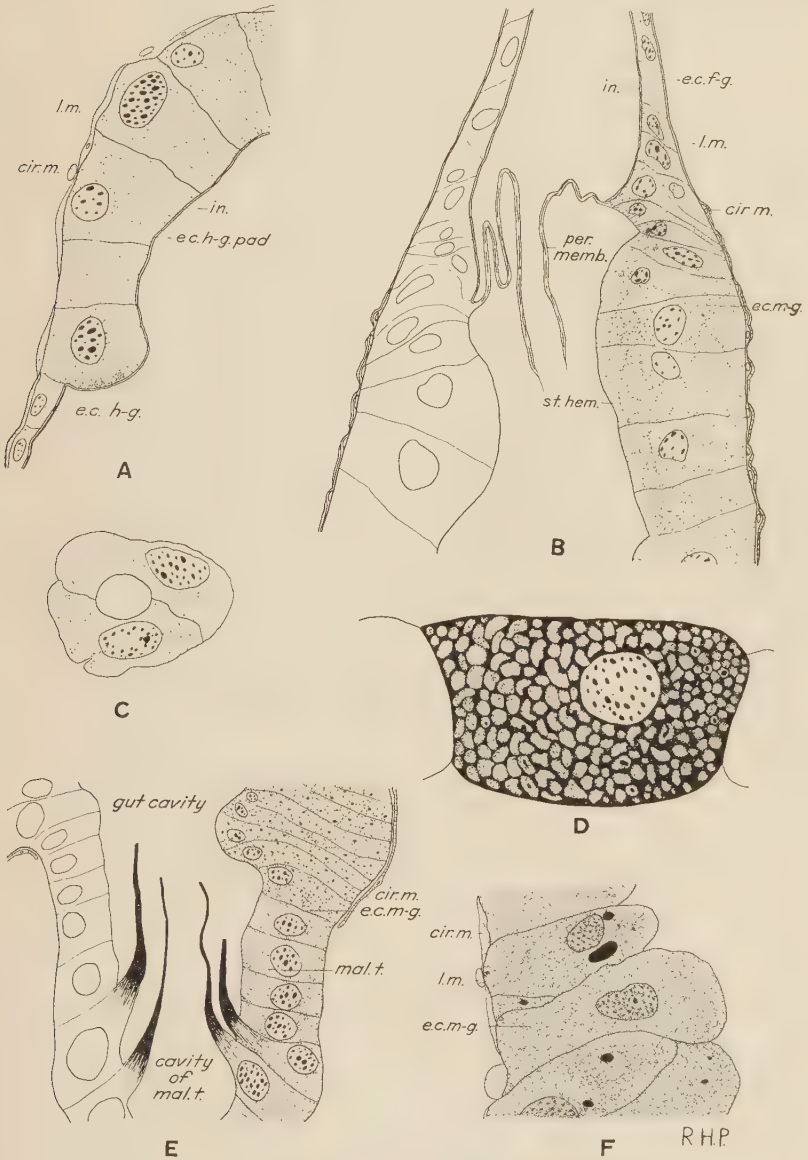

FIGURE 2.—Anatomical details of the cotton flea hopper, all about × 893. A, Longitudinal section of cells of hind-gut pad and hind-gut wall; *cir. m.*, circular muscle; *e. c. h-g.*, epithelial cell of hind-gut; *e. c. h-g. pad*, epithelial cell of hind-gut pad; *in.*, intima; *l. m.*, longitudinal muscle. B, Longitudinal section of esophageal valve; *cir. m.*, circular muscle; *e. c. f-g.*, epithelial cell of fore-gut; *e. c. m-g.*, epithelial cell of mid-gut; *in.*, intima; *l. m.*, longitudinal muscle; *per. memb.*, peritrophic membrane; *st. hem.*, striated hem. C, Cross section of one of the anterior accessory salivary glands. D, Longitudinal section of a cell of the anterior salivary gland. E, Cross section of a part of the pyloric valve, showing entrance of a Malpighiantubule; *cir. m.*, circular muscle; *e. c. m-g.*, epithelial cell of mid-gut; *mal. t.*, Malpighian tubule. F, Cross section of several cells of the mid-gut wall; *cir. m.*, circular muscle; *e. c. m-g.*, epithelial cell of mid-gut; *l. m.*, longitudinal muscle

cells found in any part of the mid-gut; they are uniform in appearance throughout unless changed by distension on account of the gut contents. Near the entrance of the Malpighian tubules the strands of muscle seem to be more numerous.

The pyloric valve is quite simple in this species. Just anterior to where the Malpighian tubules enter the alimentary tract a ring, two cells thick, of the enteric epithelium projects inward and upward. Between this place and their entrance the mid-gut is somewhat constricted. There seem to be no other structures of note in the valve. The structure of the entrance, however, shows some things of particular interest. The four Malpighian tubules join the gut at about equal distances around its circumference. Five or six cells back from the entrance of each tubule there are two circles of cells which give rise to chitinous projections, having much the appearance of the "flame cells." (Fig. 2, E.) Unlike the intima of the hind-gut, these projections stain a deep black with iron haematoxylin, as in the heavily chitinized stylets and pharyngeal plates. The projections have the appearance of being definite secretions of each of the cells and are long enough to extend out through the opening of the tubules and back into the lumen of the hind-gut.

The wall of the hind-gut, except for the hind-gut pad, shows very little difference in structure throughout its entire length and almost no external morphological differences. This portion of the alimentary canal extends from the opening of the Malpighian tubules to the anus, which opens on the ventral part of the last segment. The posterior part of the hind-gut is often somewhat constricted to form a kind of rectum, but there is no difference in the character of the wall. The hind-gut pad consists of a circular disk of tissue surrounding the pyloric valve just below the entrance of the Malpighian tubules and lies mostly on the dorsal side of the hind-gut. (Fig. 1, A.) The cells which compose it are relatively very large and contain large basal nuclei. (Fig. 2, A.) The cytoplasm is fibrous on the distal two-thirds of these cells and has a striated appearance under the microscope. The chitinous intima is somewhat thicker here than in the remainder of the hind-gut. The function of these cells is problematical. The remainder of the wall of the hind-gut consists of small cells similar to but more delicate than those of the fore-gut, with chitinous intima on the inside and a latticework of circular and longitudinal muscles on the outside. These muscles extend over the hind-gut pad. The circular muscles here are on the outside.

SALIVARY APPARATUS

The salivary glands are two roughly spindle-shaped structures lying on each side of, and dorsal to, the beginning of the mid-gut. A constriction, and internally a partition, divides each into an anterior and a posterior part. The size of the gland varies greatly in different specimens, apparently due to the increase or decrease of secretion and its subsequent use. When the glands are distended with the salivary secretions they extend even into the head above the brain and far back into the abdomen. The more usual conditions are represented in Figure 1, A and B. Fastened to the anterior end of each gland is a slender muscle which attaches it to the exoskeleton on each side above the brain. The cells of the salivary gland are very large, roughly hexagonal to square, with large nuclei.

(Fig. 2, D.) The cytoplasm takes a very dense stain with haematoxylin or other nuclear stain. The nuclei are lighter but contain darkly stained granules. In the dense cytoplasm are areas which are much lighter or even clear. When the gland is greatly distended the cells lengthen and widen, but are thinner, so that the nuclei protrude out into the lumen, though they are still covered with a thin layer of cytoplasm. This gives these cells a striking appearance; they differ greatly in general outline from the same cells when the gland is not distended. The secretion in the cavities shows up prominently in sections and is different in the two divisions. Throughout, the secretion is characterized by small droplets embedded in a material which takes up a stain. In the anterior part the secretion takes a nuclear stain; in the posterior part the secretion stains as does the cytoplasm. In addition the anterior part contains some larger vacuolated droplets which sometimes hang together in chains and have the general appearance of growing yeast cells. Although appearing to be without structure, they stain darker than the remaining secretion. (Fig. 3, A and B.) These vacuolated droplets are never seen in the posterior part of the salivary gland.

The salivary duct opens into both divisions of the salivary gland from the ventral side in the region of the partition. The exact course of the duct varies considerably in different specimens, but usually it is as shown in Figure 1, B. From its opening the duct passes forward dorsally to the alimentary tract to about the anterior end of the salivary gland, then posteriorly along the side of the mid-gut to a point about even with the middle of the posterior gland. Here it makes several coils upon itself, is joined by the accessory salivary glands, and passes forward toward the head again, around the circumesophageal commissure and beneath the optic nerve. Under the pharynx it is joined with the duct on the other side, which, in a very short distance, empties into the salivary pump. (Fig. 1, C.) In sections the salivary duct has the appearance of an intracellular tube and is lined with a chitinous membrane or has here at least a very thick cell wall. In longitudinal sections the nuclei are seen to alternate so that the duct forms a sinuous tube among them. In cross section the nuclei appear decidedly eccentric. (Fig. 1, H.) They are much fewer in number as the duct approaches the salivary pump. The cell walls are indefinite.

Three of what appear to be accessory salivary glands or reservoirs join the duct in the position shown in the figure (fig. 1, B), as previously described. Two of these tubular accessory glands pass forward and are often wound about the salivary duct; the third extends posteriorly for a distance varying in different insects and is frequently enlarged greatly to form a thin-walled disklike sack which is closely applied to the mid-gut. The other branches also vary somewhat in size and shape. In cross section these accessory glands usually have several cells and more than one nucleus visible in the same section. The cytoplasm of these cells (fig. 2, C) usually contains vacuoles and is much less dense than that of the cells of the salivary duct proper, which sometimes seem to have a pigmented area around the periphery. The center of these accessory glands rarely contains any secretion which stains definitely.

The salivary pump, which connects with the united salivary duct, lies immediately beneath the anterior end of the pharyngeal pump.

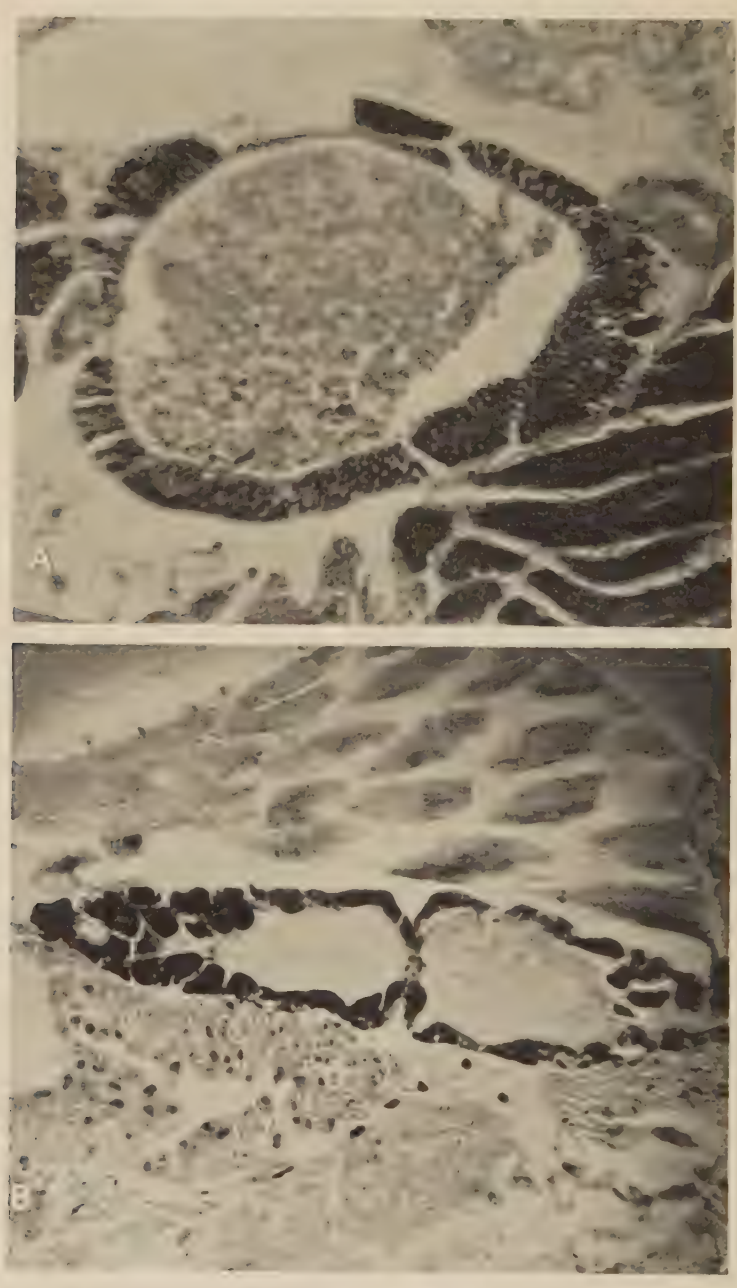

FIGURE 3.—A, a longitudinal section of part of the anterior part of the salivary gland of Psallus, showing the character of the secretion. Iron haematoxylin and orange G; B, a longitudinal section of the salivary gland of Psallus, showing the nature of the secretion of both parts. Ehrlich's haematoxylin and orange G.

It consists of a bell-shaped chitinous organ fitted against the proximal end of the hypopharynx. In the base or proximal end of the bell-shaped part is inserted the plunger of the pump, as shown in Figure 1, C. A chitinous tendon extends through the mass of muscles which work the plunger. These muscles at their middle are divided into two sections, each of which fastens to the head capsule just beneath the eyes. The salivary duct passes forward ventrally and empties into the front of the pump chamber. The dorsal lip of the opening of this duct probably acts as a valve when the plunger goes down, thus forcing the secretion out through a channel in the hypopharynx. A projection from the dorsal wall of the pump extends forward toward this efferent salivary duct and may act in some manner as a valve. The tip of the hypopharynx carrying the efferent salivary duct extends down into the ejection canal formed by the maxillae.

MECHANISM OF MOUTH PARTS USED IN PIERCING AND SUCKING

The mechanism of piercing and sucking has been thoroughly discussed by Grove (*4*) in a paper on *Psylla mali*, in which the various theories of previous writers as to how these things are accomplished have been thoroughly reviewed. Many of his statements on this homopteran may be applied here, except that the actions in this case are less involved. The insect by preference feeds on the more tender parts of the plants and, in the case of cotton, often chooses the red pigment glands. When the insect is ready to feed it elevates the front of the body and brings the beak perpendicular to the plant surface. The area is explored with the tip of the labium, and then the head is forced toward the surface of the plant. The labium bends caudad with the second joint from the base, forming at the point of the "V" an angle of about 30°. The stylets form the front of this V and extend through the other two sections of the labium, which, according to the writer's observations, never release them. The stylets are removed from the plant merely by raising the head, and another place for feeding is quickly chosen. As a rule the insects do not feed long in one place, but occasionally one is seen to remain in position several minutes. Often what are evidently punctures for exploration are made, and the stylets are driven in only a short distance and withdrawn. The entire beak is quite flexible, and a surprisingly large area can be investigated by means of the tip of the labium without further movement by the insect. In a study of sections of the plants on which the insects have fed, it has been noticed that the beak rarely penetrates farther than the first cells of the xylem.

The action of the salivary pump is relatively simple and in this insect is similar to that described for other Hemiptera. The large salivary pump muscles serve to pull back the plunger, thus creating a partial vacuum in the chamber and drawing in the salivary secretion. The elasticity of the chitinous membrane at the juncture of the plunger and the pump wall proper is probably sufficient to force the plunger back into place, close the inlet of the salivary duct, and force the salivary secretion out into the ejection canal through the hypopharynx. The secretion then passes out through the ejection

canal of the stylets. In life these insects may often be seen with a drop of semiopaque liquid, which is probably the salivary fluid, at the end of the beak.

The factors involved in drawing out the sap from the host plant along the suction canal, as they have been stated by Grove and others, may be briefly reviewed thus: (1) Capillary attraction up through the suction canal; (2) pressure of the sap or turgidity of the cells; (3) the pumping action of the pharynx accomplished by the raising of the dorsal pharyngeal plate, probably in a series of waves starting at the anterior end; (4) the peristalsis in the esophagus; and (5) the action of the esophageal valve. Although there seems to be no definite organic connection between the end of the pharynx and the suction canal, it seems probable from the size of the muscles and from the constancy of their appearance throughout the order that the pumping action of the pharynx is the principal factor involved. It is possible that the direction of the pumping force may be reversed, driving a secretion out of the gut into the plant in addition to the salivary fluid; but from the presence of a definite esophageal valve, although this is somewhat reduced in complexity, and from the lack of visible secreting cells of any size in the fore-gut, this would not appear to be a normal occurrence in this insect.

PARASITES

During the course of the study of the anatomy of the insect, parasites have been looked for which might possibly cause the deformation of the plant tissue. By making a smear of the entire mid-gut as soon as it is removed from the insect, it is possible to find a few coccus forms of bacteria. These are relatively scarce and are no more than might be expected, having been described in other insects in the same situation, and their presence here is probably not of importance in the general problem under discussion. Jenner's blood stain was used on these slides. The presence of some dark staining bodies has been described in the enteric epithelium of the mid-gut. (Fig. 2, F.) Three things with regard to these seem to point to their being metabolic products rather than parasites: (1) They vary greatly as to size and shape; (2) it is impossible to demonstrate anything but a homogeneous composition by means of stains; and (3) these bodies are found in the lumen of the mid-gut and hind-gut from the point where they begin to occur in the epithelium, about opposite the first abdominal segment, on through to the end of the gut in apparently undiminished size and number. It is suggested that these may be excretion products from these cells. The character of the secretions of the salivary gland in this insect has been described previously and presents some points of interest. The appearance of the smaller emulsoid bodies throughout the cavity of both the anterior and posterior division of this gland probably may be taken as the natural appearance of the staining reaction of the secretion in this insect. The appearance of the large globules in the anterior salivary gland is striking and peculiar in that it is limited to this section of the gland and was present in practically all the specimens examined, some 60 in all. (Fig. 3, A and B.)

STUDIES OF PRESERVED PLANT MATERIAL WITH RESPECT TO THE EFFECTS OF THE BITE OF THE COTTON FLEA HOPPER

NATURE AND EXTENT OF COTTON FLEA HOPPER INJURY ON COTTON

So far as the external appearance goes, the effect of the Psallus bite upon cotton is threefold: (1) It causes a shedding of the young flower buds, or squares; (2) the number of internodes is increased while their length is decreased; and (3) conspicuous swellings appear at intervals over the whole plant but seem to occur either near, or exactly at, the point of the bite, the number of these bites and the persistence of the swellings giving rise to the apparent systemic condition. As a result of the first two, the entire habit of the plant is much changed, so that "hopper plants" may easily be recognized in any field. These plants are further characterized by having short branches excepting the lower two or three, which are often long; the plant itself is tall and spindling. The swellings appear on all parts of the stem, petiole, midrib, and the main veins of the leaf.

EXPERIMENTS ON HOPPERS AND ON LIVING COTTON PLANTS

During the summer of 1925 a number of experiments were undertaken at Port Lavaca, Tex., to determine whether the insect carried a plant disease or, if not, what were the main factors producing the result of the insect bite. Since material from these experiments has been used in the present study it is necessary to review the results of this work.

The experiments were principally attempts to duplicate the work of the hopper by artificial means. As the summer's work advanced it became more evident that the effect of the hopper's bite was quite local and apparently not transmitted through the plant itself from one part to another. So, while the first problem was kept in mind, the later work was directed toward the isolation of other possible factors involved in the production of abnormal plants as a result of hopper bite.

INOCULATIONS AND FIELD EXPERIMENTS

Methods

The plants used in all experiments were chosen in the field. A certain amount of protection against hoppers was afforded by dusting the plants with sulphur, and in certain experiments by covering them with sacks. The work was somewhat handicapped in some phases by the lack of plants entirely free from all insect injury. The large number of plants used made it impossible to provide cages.

At first, three methods of inoculation were used: (1) Hypodermic injections in various parts of the plants; (2) cuts with a safety-razor blade dipped in various solutions; and (3) punctures with a dissecting needle likewise dipped in the solutions. Later, owing to the fact that the mechanical disturbance of the plant tissue obscured the effect of the solutions, these methods were largely replaced by the use of punctures made with pins known as "minuten Nadeln." The pins were set in a small piece of softwood, mounted on a handle for convenience in using, the tip of the handle being dipped in shellac to fix the pins in place. After each set of inoculations all instruments used were sterilized in boiling water and 90 per cent alcohol, and the minuten Nadeln apparatus was again dipped in shellac. The places of inoculation and other special methods used are described under

each experiment. In all cases where hoppers were used in the experiments, from 100 to 150 of the insects were crushed in about 3 c. c. of rain water.[6] In most cases notes were taken at 24-hour intervals.

The First Series of Experiments

G. F. White and K. P. Ewing started the first series of experiments with 70 cotton plants before the writer's arrival. Later some of the inoculations and notes were made by the writer. In these experiments the razor blade, dissecting needle, and hypodermic needle were used on leaf veins, leaf petioles, square stems and branches, the main stem at various places, and the root crown. Suspensions of crushed hoppers, croton juice, crushed diseased cotton, and crushed mint were injected. None of the results were clear-cut, but some differences in the way the lesions healed were noticed. There was no evidence of any systemic disturbance of the plant or any more shedding of the squares than occurred among the checks, hoppers being present in small numbers in the field.

The Second Series of Experiments

In the second series 60 cotton plants were inoculated in a series of 5 experiments of 12 plants each, solutions from croton, mint, and diseased cotton juice, and suspensions of crushed hoppers from croton and from cotton being used. Inoculations for each experiment were as follows:

Plant 1 (check): Water injected with a hypodermic needle.

Plants 2, 3, and 4: Solution injected with a hypodermic needle into the base of the terminal bud and on two upper square stems.

Plant 5 (check): Safety-razor blade dipped in water, and slits made in two upper leaf petioles and two upper square stems.

Plants 6, 7, and 8: Razor blade dipped in solution, and inoculations made in places similar to those of the check.

Plant 9 (check): Pin punctures (minuten Nadeln), two groups of three on each of two upper leaf petioles and on a second square stem, and one group of three on the upper square stem, the groups of three being a stated distance apart, the pins having been dipped in water.[6]

Plants 10, 11, 12: Pin punctures made as in check; pins dipped in solution.

None of the plants, except those treated with crushed hoppers from croton, showed any constant, definite distinction between the checks and the inoculated plants. In the case of the checks injured with a razor blade the cuts were clean and healed with a tan color. In the plants inoculated with hoppers from croton there was a growth of distinctive whitish cells from the sides and bottom of the upper leaf petiole of one, the lower leaf petiole of another (slight), and from all the cuts on a third plant. In the case of the pin punctures the checks showed dark holes at the points of puncture, sometimes with a slight swelling about them. In the plants inoculated with hoppers certain punctures became enlarged and burst open, showing a tan-colored

[6] Distilled water free from metallic impurities was not available where the experiments were carried on. In view of this fact and in the absence of sterile plants in the field, it was necessary to depend on a difference in reaction between plants inoculated with rain water and those inoculated with other substances in rain water, rather than to expect the checks to be entirely free from injury. Rain water was therefore used throughout the experiments.

granular tissue beneath the skin. These slits extended sometimes for a millimeter or more on each side of the puncture. This condition appeared in many of the punctures on all three plants inoculated, especially on the leaf petiole and second square stem. The slits appeared between 48 and 72 hours after the time of inoculation. A single puncture on one of the plants inoculated with mint had a somewhat similar appearance. These developments are referred to in this paper as split lesions.

Injections of Suspensions of Cotton Buds on Which Hoppers Had Been Caged

A hundred hoppers were caged on the extreme tip of each of three cotton plants for 24, 48, and 72 hours, respectively. At the end of that time the tips were removed, crushed in 1 c. c. of water and injected into 10 plants by pin punctures in the two upper leaf petioles and two upper square stems. A fourth plant was protected from hoppers for three days, examined and found free from hopper damage, and was crushed and injected as a control. The results are given in Table 1.

Table 1.—*Effect on cotton plants of injections of rain water containing soluble matter from crushed tips of cotton plants on which Psallus had been feeding, Port Lavaca, Tex., July, 1925*

[Ten plants were inoculated on each date and 21 inoculations were made per plant]

Date inoculated	Period tip was exposed to feeding of Psallus	Average height of plants July 10	Plants showing split lesions	Total split lesions	Average plant growth during—	
					First week after July 10	Second week after July 10
1925	*Hours*	*Inches*	*Number*	*Number*	*Inches*	*Inches*
July 7	24	18.05	8	40	4.2	4.75
July 8	48	19.95	10	45	3.2	4.85
July 9	72	17.15	10	62	3.4	4.55
Do	(a)	20.65	7	16	4.4	4.40

[a] Check tip; suspension was made from an uninfested tip.

In addition to the split lesions, similar to those caused by inoculation with crushed hoppers, there was a difference in the average rate of growth which was paralleled by results with hopper cages; i. e., a slowing up of the growth when hoppers were first introduced, followed by a slight increase.

Effects on Cotton Plant of Continuous Exposure to Suspensions of Crushed Hoppers and Hoppers Caged on Localized Areas

Eight cotton plants in the field were protected from hoppers for over a week. After the experiments were started, however, some hoppers hatched out in two of the plants and caused hopper squares [7] before they were found and killed. Cups of adhesive plaster were made about the stems of three of the plants; one of these was filled with water to serve as a check, two were filled with crushed hopper suspension, and slits were then cut in the stem below the level of the

[7] The term "hopper squares" is used to designate those squares which when very small darken and die in a way which is typical of the injury produced by *Psallus seriatus* and related insects on cotton.

liquid so that the plants might continually take up the solution from the cups. The liquid remained about the check and one treated plant for 12 hours and about the other plant for 48 hours. Two plants, one of them the check, on which live nymphs were found, showed hopper squares. The other plant showed no typical hopper squares. The pressure of the sacks over the plants may have caused some of the squares to die. Table 2 summarizes the results.

TABLE 2.—*Effect on cotton plants of exposing the stems to suspensions of crushed cotton flea hoppers, applied July, 1925, Port Lavaca, Tex.*

Plant No.	Period of exposure	Height at time of inoculation	Growth during—		Dead squares found during the experiment
			Week preceding inoculation	Week following inoculation	
	Hours	*Inches*	*Inches*	*Inches*	*Number*
104	12	20.5	6.5	3.0	0
105 (check)	12	25.0	6.5	3.5	1
106	48	23.0	7.5	2.5	[a] 6

[a] The pressure of the sack over the plant may have caused some of the squares to die.

Five plants were used in a test to determine the effect of hoppers confined on a limited area of the plant. One of these was used as a check, and on the other four were fitted vials from which the bottoms had been removed. Into these vials 15 hoppers were introduced every other day and there confined so that they could feed only on the area beneath the vial. The vials were placed as close to the terminal bud as possible. Data concerning these plants are given in Table 3.

TABLE 3.—*Effect of confining cotton flea hoppers on limited areas of cotton plants, Port Lavaca, Tex., 1925*

Plant No.	Height of plant at beginning of experiment, July 10	Total growth of plant from July 10 to 17	Distance from center of vial to tip of plant on July 17	Growth of plant from July 17 to 24, measured from center of vial to tip	Total growth of plant from July 24 to August 1 [a]
	Inches	*Inches*	*Inches*	*Inches*	*Inches*
107 (check)	20.5	4.0		[b] 5.00	1.50
108	19.5	3.5	1.3	1.65	1.05
109	13.0	6.5	1.0	1.00	0
110	11.5	3.5	1.9	1.80	1.50
111	19.0	5.0	2.6	2.10	.50

[a] Limited growth is probably due to pressure of inclosing sacks.
[b] Total growth of plant in this one case.

The plants showed absolutely no hopper squares and little, if any, difference in growth. The only evidences of the presence of the hoppers were the lesions beneath the vials.

CHEMICAL INTRODUCTIONS AND INOCULATIONS FOR THE STUDY OF LESIONS ON COTTON STEMS

Commercial diastase, pepsin, trypsin, and invert sugar sirup solutions were each introduced into five plants. On each plant the introductions with minuten Nadeln pins were in two groups of three on each of the two upper leaf petioles and second square stem and one

group of three on the upper square stem. The checks were five plants similarly treated with water. At the same time, partly to serve as checks and partly to serve in a study of lesions, five plants were inoculated from 17 to 21 times with crushed-hopper suspension, in groups of three each at intervals of about one-half inch. Table 4 gives a summary of the two experiments.

TABLE 4.—*Effect produced on cotton plants by injections of various chemicals and of crushed cotton-flea-hopper suspensions, Port Lavaca, Tex., 1925*

Material injected	Injections per plant	Plants showing split lesions	Total lesions	Average height of plants at beginning of experiment	Average growth per plant during first week after treatment
	Number	*Number*	*Number*	*Inches*	*Inches*
Not injected (check plants)	21	2	2	12.5	4.7
Diastase	21	5	30	10.1	3.6
Pepsin	21	3	11	10.6	3.2
Trypsin	21	1	2	11.3	3.9
Invert sugar	21	0	0	13.5	3.1
Not injected (check plants)	54–63	3	[a] 28	14.7	3.2
Crushed-hopper suspension	54–63	5	111	12.2	2.9

[a] Nearly all on one plant.

Diastase was the only reagent which showed any results comparable with those from the inoculation of crushed hoppers. With regard to the other plants, nearly all of the split lesions were in the third, fourth, and upper part of the fifth internodes below the bud; in other words, at about the place where the cells were ceasing to grow. This method of inoculation on the main stem seemed best.

Inoculations of Centrifuged Croton Juice

A large number of tips of *Croton engelmanni* were crushed in water and centrifuged for five minutes. Only chloroplasts and particles of other plant tissue were thrown down. The yellowish liquid above was drained off, and five cotton plants were inoculated with it on the main stem at intervals of about 1 cm. Five plants were similarly inoculated with the thicker part of the solution. None of these plants showed a single lesion comparable with those caused by the crushed-hopper suspension.

Inoculation of Croton Plants

A crushed-hopper suspension was used in inoculations on the main stem of four croton plants. The plants showed a slight darkening in the region of the inoculation, but neither swelling nor split lesions.

Inoculations of Cotton Plants with Suspensions of Crushed Hoppers

A large number of hoppers were crushed in water and that filtered through a Berkefeld filter No. W–20, 10½; the filtrate was then introduced into the main stems of five cotton plants. The residue was introduced, unfiltered, into the main stems of five other cotton plants. In order to determine the effect of letting the suspension stand for some time before inoculating with it, five plants were

inoculated in the main stem with freshly crushed-hopper suspension and three hours later five more plants were inoclutaed with the same suspension. The results of these two experiments are presented in Table 5.

TABLE 5.—*Number of split lesions produced by inoculating cotton plants in various ways with suspensions of crushed flea hoppers, Port Lavaca, Tex., 1925*

Material injected	Plants	Inoculations per plant	Split lesions
	Number	*Number*	*Number*
Filtrate from crushed hoppers [a]	5	54–63	7
Residue left after filtering the suspension of crushed hoppers [a]	5	54–63	45
Unfiltered suspension of crushed hoppers [a]	5	54–63	36
Suspension of crushed hoppers, 3 hours after preparation	5	54–63	3

[a] Freshly prepared.

It is noticeable that the number of split lesions was less on these plants which were large than on the younger plants used earlier in the season from this same patch with the same number of inoculations. (Table 4.)

Intermittent Inoculations

This experiment was started as soon as the late-planted young cotton began to put on squares. From August 7 to September 11, 10 plants were given twice a week from 3 to 9 inoculations with crushed hoppers on the two upper internodes of the main stem and on each branch. Five plants were similarly treated with water. These 15 plants and 5 others for comparison were measured twice weekly. During the first week in September the inoculations were discontinued on 1 check plant and on 4 of the other plants to test the recovery of the plants from the effect of the inoculations. Toward the end of the experiment from about 150 to 200 inoculations per plant were given twice a week. Table 6 shows the differences in plant heights. Four plants were eliminated during the experiment, owing to injury by the bollworm (*Heliothis obsoleta* Fab.).

TABLE 6.—*Effect on growth of cotton plants of repeated inoculations with crushed flea-hopper suspensions during fruiting period, Port Lavaca, Tex., 1925*

Treatment of plants	Plants treated	Average height of plants		Growth of plants from Aug. 28 to Sept. 11
		Aug. 7	Sept. 11	
	Number	*Inches*	*Inches*	*Inches*
Check (untreated)	5	6.9	31.0	9.4
Inoculated Aug. 7 to Sept. 11 with suspension of crushed hoppers	3	7.5	23.6	7.0
Inoculated Aug. 7 to Sept. 11 with water	3	7.5	24.5	6.6
Inoculated with suspension of crushed hoppers Aug. 7 to Sept. 1; recovery Sept. 1 to Sept. 11	4	7.6	25.3	8.0
Inoculated with water Aug. 7 to Sept. 4; recovery Sept. 4 to Sept. 11	1	7.5	25.5	8.0

In addition to differences in height, all the inoculated plants showed an average shortening of the internodes by about one-third of their length. All plants made a low scrubby growth with numerous branches, often two at each internode. In all respects save in the

appearance of lesions there was no difference between the crushed-hopper and the check inoculations. Hopper squares appeared on all plants in about equal numbers. There is little doubt that in all cases this injury of the squares was due to the presence on the plant of hoppers and other mirids.

The results of the experiments reported in Tables 1 to 6 may be summarized as follows: (1) Lesions could be studied best when inoculations were made up and down the main stem at short intervals; (2) there was no evidence in any experiment of persistent systemic disturbance transmitted very far from the point of inoculation; (3) positive results were two, the occurrence of swellings at the points of inoculations with a split showing peculiar abnormal tissue later on, and a shortening of certain of the internodes which were inoculated when young; (4) the latter occurred about as often in control plants as in inoculated ones and was least common on vegetative branches, like those coming out near the bottom of the plant; (5) swellings followed by split lesions occurred rarely on the control plants and were of some significance as they appeared similar to lesions caused by the hopper; (6) these split lesions occurred in numbers on cotton plants receiving inoculations with (*a*) crushed-hopper suspension, (*b*) crushed cotton tips on which the hoppers had been caged in large numbers, and (*c*) diastase solution; (7) a split lesion consisted of a decided swelling appearing around the puncture which about 48 hours thereafter split, sometimes as much as 4 mm., exposing a tan granular material beneath, which became darker with age; (8) so far as was visible externally, plants showed a complete recovery from any and all inoculations, except in the region of lesions, which did not develop further; (9) there was a decided variation in the response of different plants, both as regards number and size of lesions and shortening of the internodes at the sites of lesions; (10) lesions appeared to develop best on internodes 3 to 5, inclusive, on the main stem, although they appeared at almost any point inoculated, that is to say, they appeared usually at the place where the cells were just ceasing their growth; (11) croton plants did not show a response similar to cotton when inoculated with crushed hoppers; (12) hoppers might be caged on cotton plants and permitted to feed close to the buds without causing a shedding of squares; and (13) suspensions of crushed hoppers which had been filtered or allowed to stand for several hours gave few or no lesions when injected into the cotton plant.

LABORATORY EXPERIMENTS

Vital Dye Experiments

An effort was made through the use of vital dyes to determine whether the hopper transferred material from one plant to another. The method used was to place the petioles of young leaves in a solution of the dye until the veins were colored, place hoppers on these leaves, and after a short time transfer the hoppers to plants which had been deprived of chlorophyll by being kept in the dark. Aqueous solutions of the following stains were used: Eosin, Bismarck brown, Congo red, aniline green, gentian violet, acid fuschin, neutral carmine, methylene blue, neutral red, and several mixtures of two of the above. All the dyes went up into the plant tissues to some extent, gentian violet least, and methylene blue, eosin, and neutral red most. During

the course of the experiments the hoppers were examined by dissection, and methylene blue, eosin, and aniline green were observed in the gut and Malpighian tubules. The green stain also caused the gut wall to appear opaque. The eosin caused the death of the hoppers in from two to three hours. Upon examination after death the dye was found in the Malpighian tubules; before death it was seen in the gut. No stain was ever seen in the second plant on which the hoppers were subsequently caged, but these experiments led to those described below.

Nature of the Effect of Hopper Bite on the Pigment Spots

In the work with hoppers and vital dyes it was noticed that places on leaves where the hoppers had fed retained, when the leaf was placed in Carnoy's solution,[8] the red pigment spots or oil glands (*9*) normally characteristic of the cotton leaf, whereas the untouched spots were dissolved out. In the experiments with this reaction the bottoms were removed from vials and replaced with stiff paper in which openings of a definite size and shape had been cut. Hoppers in varying numbers were placed in the vials for varying lengths of time, while the opening which had been made in the paper bottom of each vial was placed successively over different parts of the plant.

The effect of the bite of hoppers on pigment spots did not spread outside the area exposed to them, no matter where the hoppers were placed. The material on which the hoppers had fed was fixed in Carnoy's and in Bouin's solution for microscopic study of the areas affected. In an attempt to determine the nature of the reaction, a number of other sucking insects feeding on cotton were caged on cotton leaves in a similar manner. The aphids and two species of Cicadellidae that normally fed on cotton gave no results similar to those from the hoppers, though relatively few of these insects were used. Three specimens of nymphs of a pentatomid and four different species of Miridae gave, however, a result that seemed identical with that produced by the hopper under similar conditions.

An attempt was made to duplicate the effect of the hopper bite artificially. Stems and leaves were punctured and treated with crushed-hopper suspension and solutions of diastase, trypsin, and pepsin, and allowed to remain overnight. When placed in Carnoy's solution none of the specimens showed a distinct preservation of the spots. Later pieces of leaves and petioles were punctured and placed bodily in suspensions of crushed hoppers and in diastase solution for varying lengths of time. The hopper suspension showed no effect, perhaps on account of its great dilution; but the material soaked four hours in the diastase solution showed the preservation of the pigment spots about the areas of punctures. The checks that had been soaked in water showed no effect. Lack of time prevented the trial of other chemicals.

Summary of Laboratory Experiments

So far as the transference of material, by regurgitation, from plant to plant was concerned, the experiments with vital dyes were negative, but further tests along this line seem warranted.

[8] Owing to lack of absolute alcohol the Carnoy's fixing solution was made up with 95 per cent alcohol 1 part, glacial acetic acid 1 part, chloroform 1 part, corrosive sublimate to saturation. The material was washed in 70 per cent alcohol.

Methylene blue, eosin, and neutral red proved to be the most promising dyes for tests of this kind.

When hoppers were caged on a leaf it was found that the pigment spots or glands about their bites failed to dissolve out in Carnoy's solution, as did the other spots.

Similar results were given by pentatomid nymphs and four other species of mirids under similar conditions.

Leaves punctured and soaked in crushed-hopper suspension gave no results, probably owing to the great dilution.

Leaves punctured and soaked in diatase solution for a short time showed a preservation of spots about the punctures when preserved in Carnoy's solution.

IMMEDIATE EFFECT OF THE HOPPER BITE

The material which was used in the study of the immediate effect of the hopper bite consisted of sections of those leaves on which hoppers had been caged for varying lengths of time and had been restricted in their feeding to a small square area by means of an opening in a paper at the bottom of a vial, as previously described. Whether the insects had fed on the plant 3 hours or 24, there were no apparent differences in the stained preparations. In the cells the cytoplasm showed a tendency to plasmolyze throughout the area on which the insects had fed. The chloroplasts and nucleus seemed to have been broken up and scattered through the cell, giving it a decided homogeneity. Iron haematoxylin stained all of the cell contents very heavily, so that it was impossible to make out details. Other stains, such as Ehrlich's triacid mixture, Ehrlich's acid haematoxylin, and safranin, gave somewhat better results but failed to resolve the cell contents into constituent parts. In this respect all the cells of the leaf in the square area on which the insects had fed reacted similarly. Cell walls in places both inside the leaf and on the outer epidermis had been ruptured in various places. These breaks had the appearance of being made mechanically rather than by any dissolving action. There seemed to be no tendency in this tissue for the effect of the bite to spread beyond the confines of the square in which the insects had fed, the cells on its edge being entirely normal.

In connection with this part of the study, sections were made of leaves which had been punctured and soaked in diastase solution for four hours. These sections in places around the punctures showed a clumping of the cytoplasm and dissolution of the chloroplasts similar to that of the material described above. The cytoplasm of these cells stained a deep black in iron haematoxylin. In one place noted about 15 palisade cells were affected on each side of the point of puncture. This may perhaps be taken as the average of spread in this length of time.

Relatively little could be ascertained concerning the effect of the insect's feeding on the pigment spots or glands. In the area not touched by the insects these appeared as circular openings surrounded by cells of an epithelial nature. In the glands on which the insects had fed or which had been touched by the diastase solution, there was a black staining material in the center of the gland and throughout the surrounding cells. The chemistry and anatomy of these glands have recently been studied by Stanford (*10*). It is interesting to note that the tissue on which the insects had fed was somewhat similar

in reaction and appearance to that found surrounding the beak of certain aphids when this organ was inserted into a plant. This has been described and figured by Wells (*12*) and Biedermann (*1, pp. 808–810*), and it has been shown that this sheath about the proboscis consists of calcium pectate and tannic substances.

A STUDY OF THE SWELLINGS AND RELATED INJURY CAUSED BY THE BITE

Externally the swellings caused by the bite itself showed little variation except as regards size, extent, and amount of splitting. On the plant they occurred on the stems, petiole, midrib, and smaller leaf veins. The swellings themselves were principally the result of an increase in size of the individual cells, and to a very slight extent of an increase in the number of cells. The swelling might take place in all directions but was always more obvious in cross than in longitudinal sections, hence laterally rather than with the axis of the stem. In the center of the swelling, apparently at the place where the insect bites, there was a distinct destruction of some of the cell elements, while others enlarged to take their place. Along the margin of an area of destruction certain cells seemed to have collapsed. Sometimes as a result of this destruction of cells, and at other times apparently without visible cause, there was a displacement of the position of normal cells. This was especially true of the elements of the vascular bundle, the position of the xylem often being changed as shown in Figure 4, A, and the constituent parts broken up as shown in Figure 4, B. The chief change in cell structure took place in the cortex and in the cells recently formed in the cambium, but the phloem was also involved, and less commonly the xylem. The individual cells showed a great diversity in form, usually being somewhat irregular and thus different in shape from those of normal condition.

In certain cases the structures of the cells themselves were changed. The epithelial cells were often found to have grown larger and varied in shape and size, appearing like those of the cortex or even more irregular. Very often certain elements had enlarged and taken the place of destroyed tissue, as is shown in the cross section of the midrib. (Fig. 5, A.)

The most marked change, however, was in the content of the cell wall about an area where the cells had been destroyed, or in a lesion which had split so that the cells were exposed to the air. Here the cellulose had disappeared from the cell walls and pectic material alone apparently survived. This was evident when such a section was examined under polarized light, and it was also shown by stains. This change in structure seemingly gave rise to the characteristic appearance of the split lesions seen in field work. In these structures the swelling split open, and the enlarged cells appeared as a tan granular substance in the wound. In section these split lesions were seen to be lined with dead cells, and the open part often extended back under the epidermis for a short distance in all directions, sometimes leaving pockets of destroyed cells overlaid by living ones. Some swellings which showed no external split had below the epidermis an extensive pocket of dead cells and often relatively large air spaces.

Frequently the entire contents of injured cells were lacking. But when still present there was always a most striking change of appearance in these parts. When stained with iron haematoxylin and

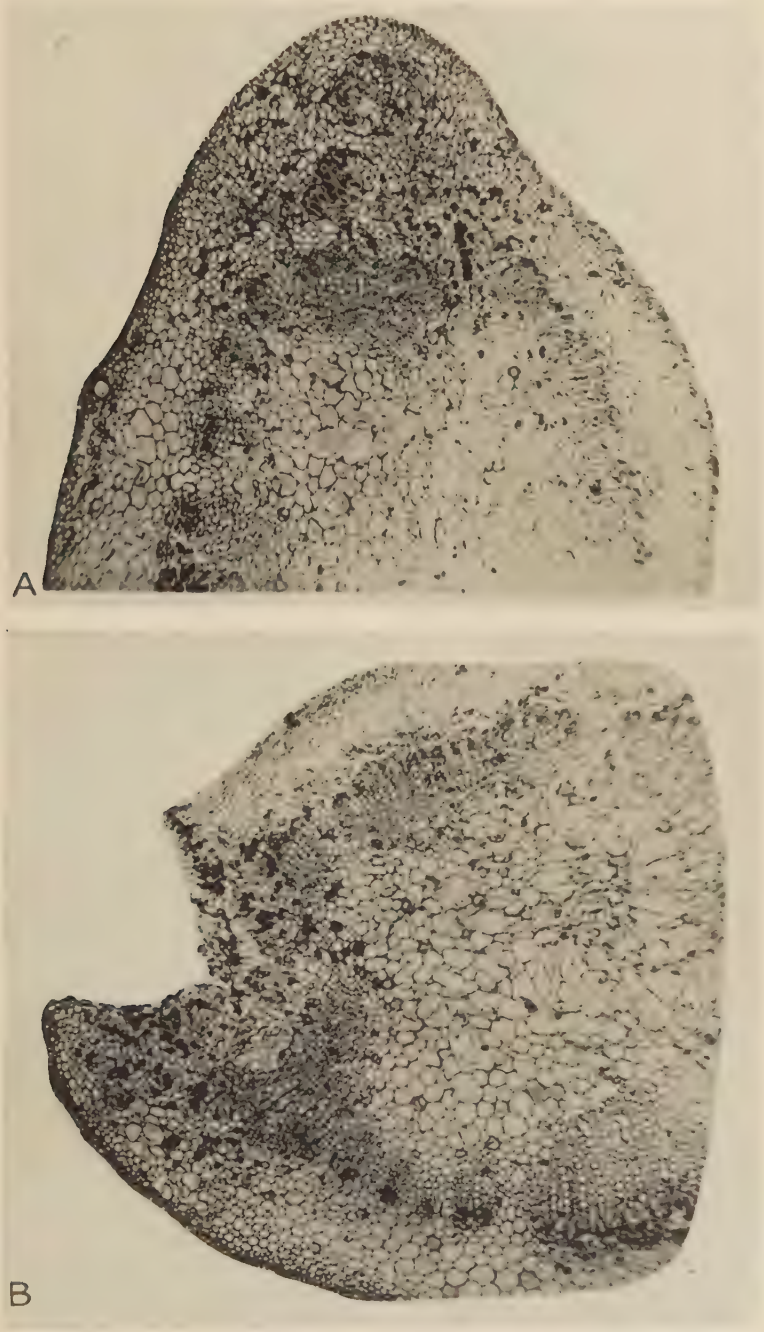

FIGURE 4.—Injuries to cotton by the cotton flea hopper, *Psallus seriatus:* A, Swelling and misplaced vascular bundle on stem caused by the insect; B, cross section of split lesion on stem caused by hopper bite. Material from hopper cages, Port Lavaca, Tex. All the photomicrographs of plant material used in the illustrations of this article were of sections stained with iron haematoxylin and orange G

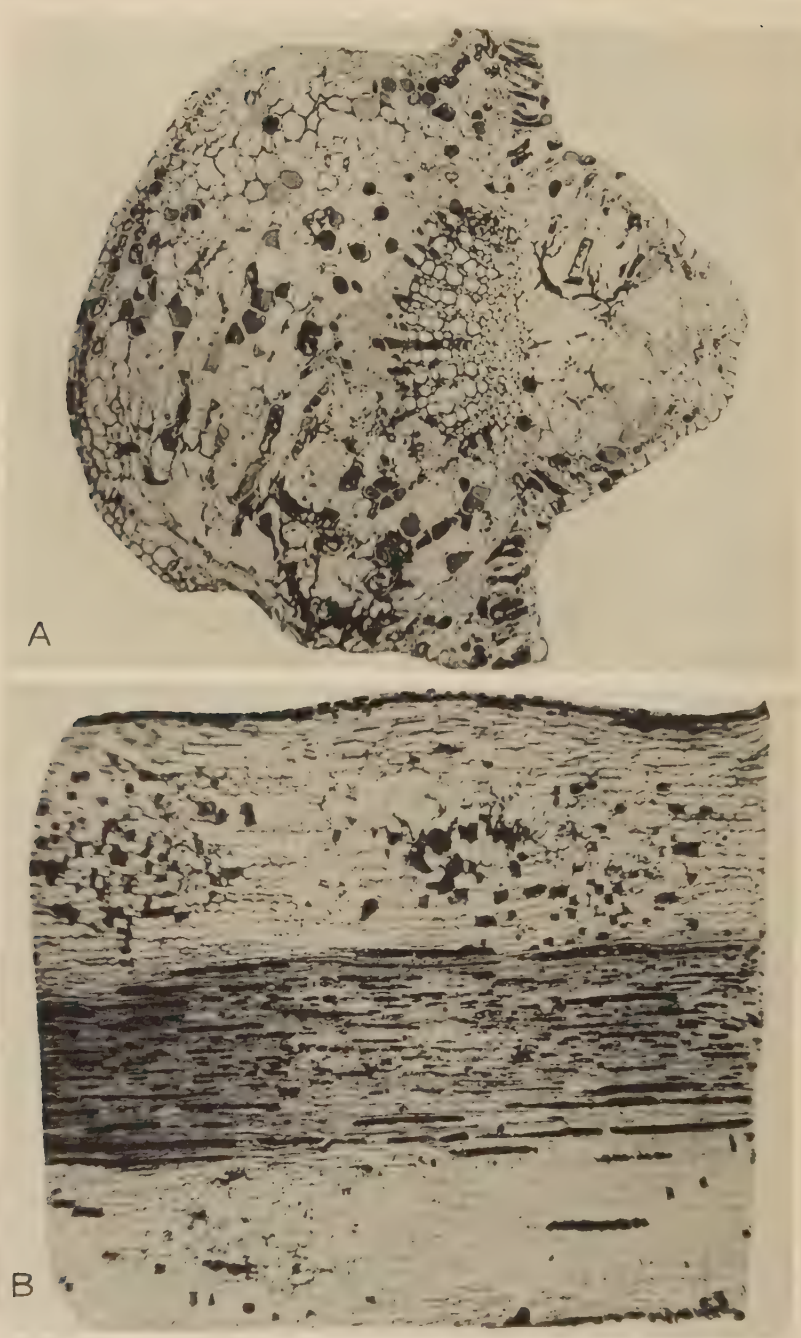

FIGURE 5.—Injuries to cotton by the cotton flea hopper, *Psallus seriatus:* A, Cross section of swelling on midrib of cotton leaf; B, longitudinal section of stem preserved four days after the hoppers had been caged on the plant, showing a series of cells containing granular material which came from near the beginning of the region of the welling. Material from the hopper cages at Port Lavaca, Tex.

orange G, the cells could be separated into four types, which seemed to merge into each other. Certain cells were filled with a homogeneous material which stained a deep black and showed no trace of nucleus or chloroplasts. Other cells were filled with a homogeneous yellow-staining material but with the nucleus still in the cell and the chloroplasts intact around the periphery. Other cells were filled with yellow particles of the same size in any one cell, but of different sizes in different cells. In certain of this group of cells a nucleus, or the chloroplasts, or both, were still visible. The last group of cells to be noted was that in which the cells contained oval or round particles, often with darker rim, and had a glistening appearance much like spores of fungi. These varied in different cells from the size of the largest yellow particles to about one-half larger. At times they were arranged in a loosely branching series throughout the cells. At other times they were arranged around the periphery of the cells. An average size for the largest was about 0.005 mm. (Fig. 5, A.)

The exact extent of the effect of the bite up and down the stem has not been ascertained. Experiments during the summer of 1925, however, point to the probability that it is fairly local, at least not extending more than two or three internodes from the point of bite. A very interesting fact concerning the material found in the affected cells about the lesions was that the cells involved tend to occur in vertical series, each series often separated from the next by several normal cells. There was a tendency at times to spread subcortically and also around the epidermis.

Material which had been inoculated with crushed-hopper suspension and later preserved and examined microscopically showed throughout effects which were similar in all cases to those due to the punctures by the hopper. The type of swelling and the change in cell structure and in cell contents were closely parallel in both cases. Figures 5, B, and 6, A, show this comparison very well. By microscopic examination it was also found that the method of inoculation with "minuten Nadeln" was quite satisfactory, the pin pricks extending only through the cortex, and hence very little farther than the proboscis of the insect entered.

SHEDDING AND INSECT INJURY

A study of the literature on abscission in cotton shows that two theories have been advanced in regard to the probable cause of the shedding of the squares and bolls. Lloyd (*7*) has brought together considerable material to support his contention that abscission is a result of competition between bolls for water. Mason (*8*) has given support to the theory that the shedding is determined by the rate at which food is elaborated by the plant and is used by the developing fruit. The exact details of the method of abscission have not been completely worked out. It has been shown, however, that abscission is preceded by a growth of cells in the abscission zone, followed by a digestion of the middle lamella and adjacent layers of cellulose wall. Pockets of resin or gum inclosing loose cells are frequently present.

It will be seen from the preceding description of insect injury that the probable nature of abscission due to the insect may support either theory, or both, or neither. It is possible that a change in the cell wall or in cell contents will start the abscission reaction. It has

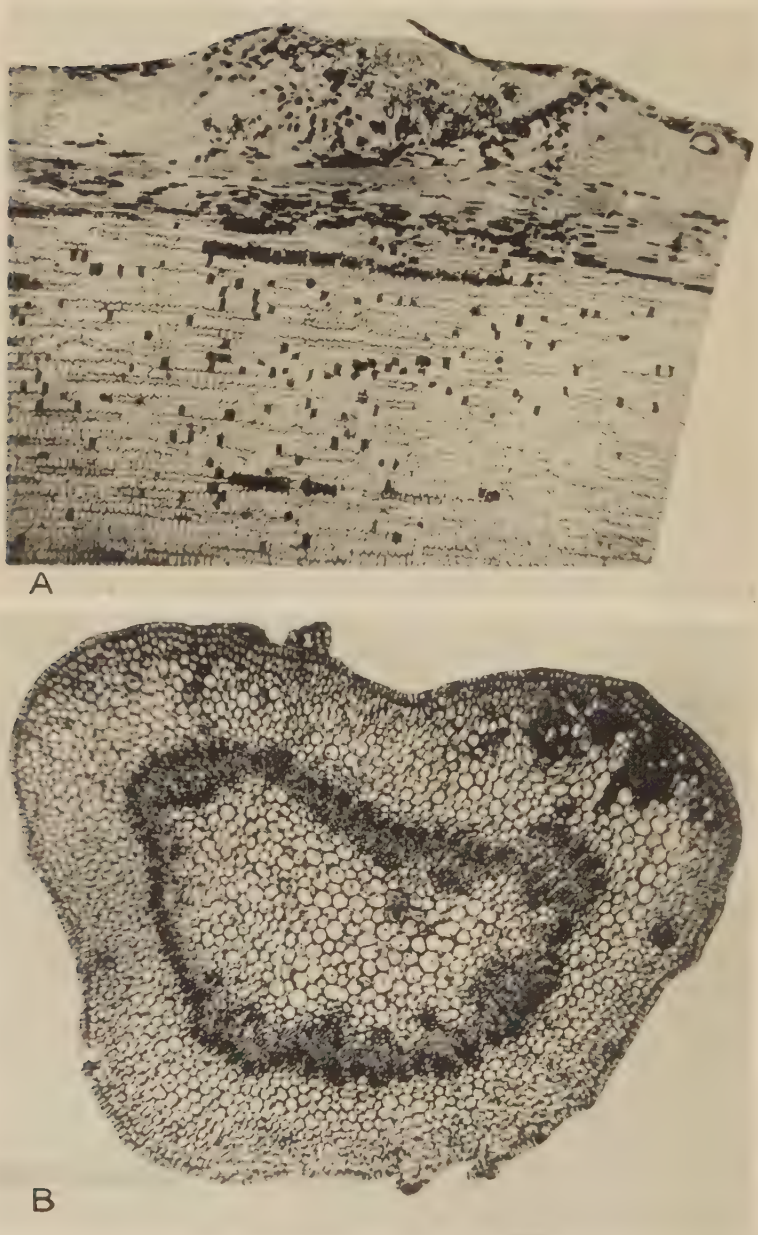

FIGURE 6.—Injury to cotton plant by the cotton flea hopper: A, Longitudinal section of stem through place of inoculation with crushed-hopper suspension, preserved 48 hours after puncture; B, cross section of stem in which the feeding place of the insect appears black, preserved after the insects had fed on the plant for 24 hours

been noted that a destruction of cells sometimes takes place at the site of the insect bite without being visible externally. This actual destruction of cells may in itself be the cause of the shedding of the squares or it may operate to prevent the absorption of water by the developing bud. The change in cell contents may have a similar result.

Preserved material has been examined to see whether it shows any correlation between the length of time during which the insects have been allowed to feed and the changes in the plant tissue. Four days after hoppers have been caged on a plant a cross section of the bud shows all stages of cell secretion, and in a longitudinal section all variations may often be seen in a single row of cells, so that it is impossible to point out any one cell as being the one in which the change originated. On one occasion insects were caged on a bud for 24 hours and then removed and the bud preserved in fixing fluid. On examination sections of this bud showed the cell contents in the condition previously described as containing yellow particles, and in other places the cells contained the black particles. (Fig. 7, A.) Where each locality represented a separate place of bite there was no admixture of the two types of cell contents. Certain cells were entirely emptied of their contents, and in others there was a clumping of the material of the cell, which stained very darkly, paralleling the condition described above. In the stem of this same bud a developing egg was found which was probably that of Psallus. The only evidence of injury in the region about the egg was a collapse of some of the cells. A typical section is shown in Figure 7, B.

CELL INCLUSIONS

In a study of this kind the possibility of the presence and transmission of organisms of a parasitic nature must be taken into consideration. The change in the character of the cell contents is the only fact derived from microscopic study which would point to the possibility of such organisms being present. It seems impossible that these peculiar structures are artifacts due to fixation, since a large number of fixing fluids have been used under various conditions. However, it may be that they are the normal regeneration responses of the plant to a wound of any kind. Other than this, there are four possible explanations for the change in appearance in the cell contents: (1) The structures seen may be gums or resins due to excess food, the products of insect secretion injected into the plant; (2) they may consist of a deposit of material by the plant in direct response to toxins or enzymes injected by the insects; (3) they may represent a developing parasitic organism showing stages of growth, and may be any of the following—yeast, slime mold, fungi, amoebae, or bacteria. In general appearance the diseased portion resembles a similar portion of the club root of cabbage. Certain stages show a likeness to developing yeast, and in this connection it is interesting to remember the similar appearance of the bodies in the salivary glands in the insect. (4) A part of the appearance may be due to a cell product induced by the developing parasite.

FIGURE 7.—A, Three feeding places of the cotton flea hopper on a bud of cotton, the plant having been preserved after the insects had fed on the bud for 24 hours; B, developing egg of *Psallus seriatus* in cotton bud, plant tissue otherwise normal

A STUDY OF FRESH MATERIAL OF THE COTTON FLEA HOPPER AND OF INFESTED COTTON

METHOD OF DISSECTION

During July, 1926, an attempt was made to get further information with regard to the exact method by which the cotton flea hopper injures the plant. It was found possible to take out entire the salivary glands of Psallus. The insects were placed on their sides in a watch glass and were completely covered with a thin layer of paraffin which covered the bottom of the glass and which had been thoroughly impregnated with powdered charcoal. It was easily possible then to cut away the chitinous body wall from both dorsum and side, exposing the alimentary canal and other organs.

The anterior part of the salivary gland is nearly transparent, with more opaque places which represent the nuclei of the cells. The posterior part of the salivary gland is usually white and opaque, and on dissection this appearance proves to be due to the secretion in its cavity, the walls of the gland being semitransparent, as in the case of the other half of the gland. Specimens have been found in which the entire gland was transparent. On the whole it is difficult to see these glands except against the black background of the dissecting dish.

TESTS FOR THE PRESENCE OF AMYLASE

In making tests for the presence of enzymes, the method used by Swingle (*11*) was followed, except that the starch solution was diluted to about 10 per cent. In the case of each test for the presence of amylase, a few drops of the mixture of starch colored with I–KI solution was placed in the center of each of two concave slides. The two glands from a single specimen of Psallus were removed, one placed on each slide and thoroughly torn to pieces. One slide was then heated until the drop of solution boiled, destroying the enzymes present. This slide was used as a control. Both were incubated at 35° C. for 48 hours. In a second test the glands were treated in a similar manner but without the addition of I–KI, and after incubation the resulting solution was tested for the presence of reducing sugars by means of Fluckiger's reagent. The tests were repeated twice, but in no cases was there any indication of the digestion of the starch. The tests may be taken as an indication of the absence of amylase, but the results should be checked in future by other methods, as the glands are extremely small. Amylase might, moreover, be secreted by one of the accessory salivary glands.

In another series of tests the insects were allowed to feed on a restricted area of the stem by means of the vials described in a preceding paragraph. Free-hand sections were cut through this part of the stem and tested for the location of the reducing sugars, glucose and fructose, by means of Fluckiger's reagent (*11, p. 215*). There was no distinguishable difference between the part of the cortex on which the insects had fed and the part which had been protected from them. Tests were made on material on which the insects had fed for 24, 48, and 72 hours, respectively. These tests seemed to show that injury to the plant was not due to a starch-digesting enzyme proceeding from the salivary glands.

EXAMINATION OF MATERIAL IN CELLS OF INFESTED PLANTS

In an effort to determine the nature of the material found in the cells of infested plants, free-hand sections were made of a number of lesions found in the field and also in cages where only one species of insect had been introduced. Affected plants taken from fields at Scott, Miss.; Tallulah, La.; and Port Lavaca, Tex.; and from cages from the two latter places, have been examined. In all cases an abundance of material similar to that noticed in the preserved plants has been found. Considerable difficulty has been experienced on account of the low visibility of the material in the cells of the fresh plants. The cells which are affected seem under the microscope to have a homogeneous grayish, opaque appearance and little or no detail can be made out. If I–KI solution, such as is used in testing for starch, is placed on the section, certain of these cells stain a homogeneous yellowish brown and others are seen to be packed full of nonstaining, round or oblong, glistening bodies of various sizes in different cells. Swellings on the midribs of leaves seem to afford the best material for a study of these objects in the cells. Their appearance does not differ, except as regards color, from that of preserved material previously described. Sections of stems which had swellings were treated with Fluckiger's reagent, and these showed a larger quantity of glucose and fructose in the area of the swelling than elsewhere. This was often concentrated in the cells which contained the granular material. Whether this is due to a peculiarity of this material or to the malformation of conducting tissue can not be determined at this time. The granular material in the cells has also been found in swellings from plants on which the tarnished plant bug (*Lygus pratensis* L.) had been caged.

EFFECTS OF THE HOPPER BITE ON PLANT CELLS

In order to study the immediate effects of the hopper bite, several vials containing the insects were fastened against the stems of protected plants and the insects allowed to feed through openings in the paper which replaced the bottom of the vial. A vial was removed on each successive day, and sections were cut out of the stem beneath it. These sections were examined both in water and in I–KI solution. The area on which the insects had fed contained many cells which were empty, their contents being replaced by air. In one case whole masses of cells were absent, but this was possibly due to injury during cutting. In all this material small oval or round bodies were found in the plant cells. These bodies were glistening and moved about freely in the cell or often in the water near a ruptured plant cell. They varied in number from one to a dozen or more per cell and seemed to increase in number with the increasing number of days on which the hopper had fed on the plant. It has not been possible to ascertain whether these bodies have a definite relation to the granular material which later appears in the cells of the plant. Sections have been examined of material on which the insects had fed from one to five days. No information regarding the origin of the granular material in the cells, however, has been gained by these examinations.

SUMMARY OF STUDIES WITH FRESH MATERIAL

Tests failed to reveal the presence of a starch-digesting enzyme in the salivary gland of Psallus, but the tests were not conclusive, and the accessory salivary glands were not tested.

Infested cotton plants from Mississippi, Louisiana, and Texas have been examined by means of free-hand sections.

In all cases material was found in the cells which resembled that found in the stained sections of plants from Port Lavaca, Tex.

Oval or round glistening bodies were found moving about in the cells of plants on which the Psallus had recently fed.

The observations suggest the possible presence of an organism which is transmitted by the cotton hopper but which does not penetrate far from the point of introduction.

This assumed organism may be transmitted by *Lygus pratensis* also.

The appearance of the cell inclusions studied suggests that of a myxomycete or slime mold, such as the one causing club root of cabbage.

GENERAL SUMMARY AND CONCLUSIONS

The primary purpose of this study is in the nature of foundation work for future studies. The anatomy and histology of the alimentary canal of the cotton flea hopper, *Psallus seriatus,* are given. The structure of the salivary glands and their accessory glands and ducts has been worked out, together with the structure of the mouth parts. The presence of bodies which may be parasites in the anterior part of the salivary gland is discussed.

The effect of the feeding of the cotton flea hopper on the plant cells has been described, and a study of the tissues of infested cotton has shown, in addition to the malformations of the cells, the presence of cell inclusions near the site of the puncture. In certain preparations these cell inclusions have the appearance of an invading or developing parasite and have been found in both fresh and preserved plant material.

The field experiments have shown that the inoculum or material injected by the cotton hopper does not spread far from the point of injury. The appearance of a systemic disturbance, sometimes observed in the infested fields, therefore, seems to be due to the multiplicity of bites, and the shedding of the hopper squares seems to be due to a bite near by.

The secondary purpose of the paper is to call attention to possible plant-disease transmission by Psallus and to present the evidence that has been gathered. Further work along this line must be done by the collaboration of a plant pathologist and an entomologist, and should include in addition a study of the feeding of other Miridae on cotton.

LITERATURE CITED

(1) Biedermann, W.
1910. die ernährung der insecten (hexapoda). neunter teil. *In* Winterstein, H., Handbuch der Vergleichenden Physiologie. Bd. 2, Erste Hälfte: [726]–902, illus. Jena.

(2) Chamberlain, C. J.
1924. methods in plant histology. 4th rev. ed., 349 p., illus. Chicago.

(3) Dufour, L.
1833. recherches anatomiques et physiologiques sur les hémiptères, accompagnées de considérations relatives a l'histoire naturelle et a la classification de ces insectes. Paris Acad. Roy Sci. Mem. . . des Savans Etrangers. T. 4, p. [131]–461, illus. Paris.

(4) Grove, A. J.
1919. the anatomy of the head and mouth parts of psylla mali, the apple sucker, with some remarks on the function of the labium. Parasitology 11: 456–488, illus.

(5) Hunter, W. D.
1924. the so-called cotton flea. Jour. Econ. Ent. 17: 604.

(6) Imms, A. D.
1925. a general textbook of entomology, including the anatomy, physiology, development, and classification of insects. 698 p., illus. London.

(7) Lloyd, F. E.
1920. environmental changes and their effect upon boll-shedding in cotton (gossypium herbaceum). Ann. New York Acad. Sci. 29: 1–131, illus.

(8) Mason, T. G.
1922. growth and abscission in sea island cotton. Ann. Bot. [London] 36: 457–484, illus.

(9) Snodgrass, R. E.
1927. the head and mouth parts of the cicada. Ent. Soc. Wash. Proc. 29: 1–16, illus.

(10) Stanford, E. E., and Viehoever, A.
1918. chemistry and histology of the glands of the cotton plant, with notes on the occurrence of similar glands in related plants. Jour. Agr. Research 13: 419–436, illus.

(11) Swingle, H. S.
1925. digestive enzymes of an insect. Ohio Jour. Sci. 25: 209–218.

(12) Wells, B. W.
1920. early stages in the development of certain pachypsylla galls on celtis. Amer. Jour. Bot. 7: 275–285, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1930

AUTOBIOGRAPHY

I, Reginald Henry Painter, was born at Brownwood, Texas, in 1901. I received my secondary school education in the Brownwood High School and entered Howard Payne College at Brownwood, Texas, in the fall of 1918. In the fall of 1920, I entered the College of Arts and Sciences at the University of Texas, and during my senior year, held a University Scholarship there. I received the degree of Bachelor of Arts in June 1922, then entered the Graduate School of the University of Texas, and received the Degree of Master of Arts in June, 1924. During my graduate study there and during the summers of 1922, 1923, and 1924, I was tutor in General and Invertebrate Zoology. In the fall of 1924 I entered the Graduate School of The Ohio State University and was Graduate Assistant in 1924-1925, and University Fellow during 1925-1926. During this time I have completed the residence requirements for the degree of Doctor of Philosophy.